REMARKS

Applicants would like to thank the Examiner for his careful reading of the subject application and the suggestions for amending the claims.

Claim amendments

Claims 1, 15, 25 and 54 have been amended to more clearly recite methods in which cells comprising the expression library are maintained under conditions in which the protein involved in GLUT 4 trafficking is expressed, and the cells are stimulated with insulin. Support for the amendment can be found, for example on page 6, lines 20-25; page 7, lines 11-14 and lines 15-28; page 8, lines 5-7; page 36, lines 17-19 and Examples 2-4 of the specification.

Claims 1, 15 and 54 have been amended to more clearly indicate that the expression library comprises DNA encoding a protein involved with GLUT4 trafficking. Support for the amendment can be found, for example, on page 6, lines 12-13 of the specification.

Claim 8 has been amended to more clearly recite a method of Claim 1 wherein the cells of a) are maintained in a media with high amino acid content. Support for the amendment can be found, for example, in Examples 2-4 and Claim 1 of the specification.

Claims 12-14 have been amended to more clearly indicate that the claimed invention is directed to expression libraries enriched by the method. Support for the amendment can be found, for example, on page 7, lines 11-14 of the specification.

Claim 25 has been amended to more clearly indicate that the expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane in cells is prepared comprising maintaining cells which comprise the expression library under conditions in which the protein is expressed, and contacting the cells with insulin, sorting cells comprising the expression library for cells with an altered proportion of GLUT4 at the cell surface upon insulin stimulation, thereby forming an expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane. Support for the amendment can be found for example on page 6, lines 20-25; page 7, lines 11-14 and lines 15-28; page 8, lines 5-7; page 36, lines 17-19 and Examples 2-4 of the specification.

Claim 56 has been amended to recite the method of Claim 55 further comprising additional steps of sorting expanded cells and expanding the sorted expanded cells in culture

wherein a final sorting results in production of clonal cells. Support for the amendment can be found, for example, on page 18, lines 5-7 of the specification.

Rejection of Claims 1-21, 25-31 and 54-57 under 35 U.S.C. §112, second paragraph

Claims 1-21, 25-31 and 54-57 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 2).

The Examiner states that the claims recite a protein that is "involved in GLUT4 trafficking and that "it appears that expression of the candidate proteins is required in order to practice the claimed invention" (Office Action, page 2). The Examiner suggest amending Claims 1, 15, 25 and 54 to "clearly indicate the candidate proteins are expressed and that their expression is linked to any observed changes in GLUT4 at the cell surface" (Office Action, page 3).

Claims 1, 15, 25 and 54 have been amended to more clearly recite methods in which cells comprising the expression library are maintained under conditions in which the protein involved in GLUT 4 trafficking is expressed, and that cells comprising the library and an altered proportion of GLUT4 at the cell surface upon insulin stimulation are sorted.

The Examiner states that in Claims 1, 15 and 54 is unclear "whether one must know *a priori* that the expression library *necessarily* comprises DNA encoding a protein involved with GLUT4 trafficking or whether the claims further encompass embodiments where one simply screens any expression library for DNAs encoding a protein that alters GLUT4 concentrations at the cell surface" (Office Action, page 3).

Applicants have amended the claims to more clearly indicate that the expression library comprises DNA encoding a protein involved with GLUT4 trafficking.

The Examiner states that in Claims 1, 15, 25 and 54 it is unclear "whether the comparison is between cells before and after insulin treatment, or between cells not expressing the protein and cells that do express the protein" (Office Action, page 3).

As indicated above, the claims have been amended to indicate that cells which comprise an altered proportion of GLUT4 at the cell surface "upon insulin stimulation" are sorted, isolated or screened.

The Examiner states that in Claims 3, 15 and 25 "it is unclear whether practicing the recited steps will necessarily identify a cell comprising a DNA encoding a protein that is involved with GLUT4 trafficking if the cells are already going to demonstrate a change in concentration of a GLUT4 protein on the cell surface" (Office Action, page 4).

Applicants studied the "cell-type specificity of insulin-regulated GLUT4 trafficking" (specification, page 32, lines 21-22). Applicants' invention is based, in part, on the discovery that:

> GLUT4 participates in a highly insulin-responsive compartment not only in the fully differentiated 3T3-L1 adipocytes employed, but in undifferentiated 3T3-L1 preadipocytes as well. ***Such a compartment is not present in all cell types***, since NIH 3T3 cells do not exhibit highly insulin-responsive trafficking. In CHO cells, highly insulin-responsive trafficking was observed only when the cells were cultured identically to 3T3-L1 adipocytes, in DMEM (specification, page 32, lines 23-25, emphasis added).

Therefore, the invention is directed to methods for identifying a protein involved in insulin stimulated GLUT4 trafficking at the plasma membrane comprising introducing an expression library comprising, or enriched for, DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane in cells; maintaining the cells under conditions in which the protein is expressed; stimulating the cells with insulin; and screening the cells for altered GLUT4 trafficking at the plasma membrane upon insulin stimulation; wherein altered GLUT4 trafficking ***upon insulin stimulation*** is indicative of the presence of a protein involved in GLUT4 trafficking at the plasma membrane. Cells which exhibit altered GLUT4 trafficking at the plasma membrane upon insulin stimulation under these conditions clearly indicate that a protein involved in GLUT4 trafficking at the plasma membrane is present those cells. In contrast, cells which do not

exhibit altered GLUT4 trafficking at the plasma membrane upon insulin stimulation under these conditions clearly indicate that a protein involved in GLUT4 trafficking at the plasma membrane is not present those cells. The inclusion of Claim 3 is not clear to Applicants, and Applicants respectfully request clarification.

The Examiner states that "there is no clear and positive prior antecedent basis for the phrase 'wherein the cells were cultured in media with high amino acid content'" in Claim 8 (Office Action, page 4).

Claim 8 has been amended to more clearly recite a method of Claim 1 wherein the cells of a) are cultured in a media with high amino acid content.

The Examiner states that "there is no clear and positive prior antecedent basis for the phrase 'The expression library prepared in the method'" in Claims 12-14 (Office Action, page 4).

Claims 12-14 have been amended to recite expression libraries "enriched by" the method.

Regarding Claim 25, the Examiner asks "[t]o what degree and compared to what is the expression library 'enriched'?" (Office Action, page 4).

Claim 25 has been amended to more clearly indicate that the expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane in cells is prepared comprising maintaining cells which comprise the expression library under conditions in which the protein is expressed, and contacting the cells with insulin, sorting cells comprising the expression library for cells with an altered proportion of GLUT4 at the cell surface upon insulin stimulation, thereby forming an expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane.

The Examiner states that the phrase "desired level of enrichment" in Claim 56 is unclear.

Claim 56 has been amended to recite the method of Claim 55 further comprising additional steps of sorting expanded cells and expanding the sorted expanded cells in culture wherein a final sorting results in production of clonal cells.

Applicant particularly point out and distinctly claim the subject matter which Applicants regard as the invention in the claims, particularly as amended.

Third Supplemental Information Disclosure Statement

A Third Supplemental Information Disclosure Statement (IDS) is being filed concurrently herewith. Entry of the IDS is respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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